Over-representation of proteins identified as disease biomarkers and their relation to post-mortem events

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Background

Tissue sampling is a major traumatic event that can have drastic effects within a very short timeframe at the molecular level resulting in loss of sample quality due to post sampling changes. It has recently been reported that the same proteins, regardless of tissue origin or species, are often found expressed differentially in various disease states, bringing into question the significance of these proteins as biomarkers. We report a remarkable overlap between proteins commonly identified as changing in 2D-GE p roteomic studies [1, 2] and those found to change post-mortem. Proteins with one or more phosphorylation sites are of particular interest since they appear to be over-represented in this group. We recommend an alternative technique to prevent these changes.

Method

Data was drawn from a range of proteomic studies in which tissue samples were heat-stabilized (Stabilizor system, Denator) or snap-frozen to create a list of 86 unique proteins reported to be changed post-mortem [3, 4, 5, 6, 7, 8]. The protein list was compared with another list of 48 proteins often occurring in 2D-GE experiments, drawn from publications of Petrak et al. [1] and Wang et al. [2] when studying disease states.

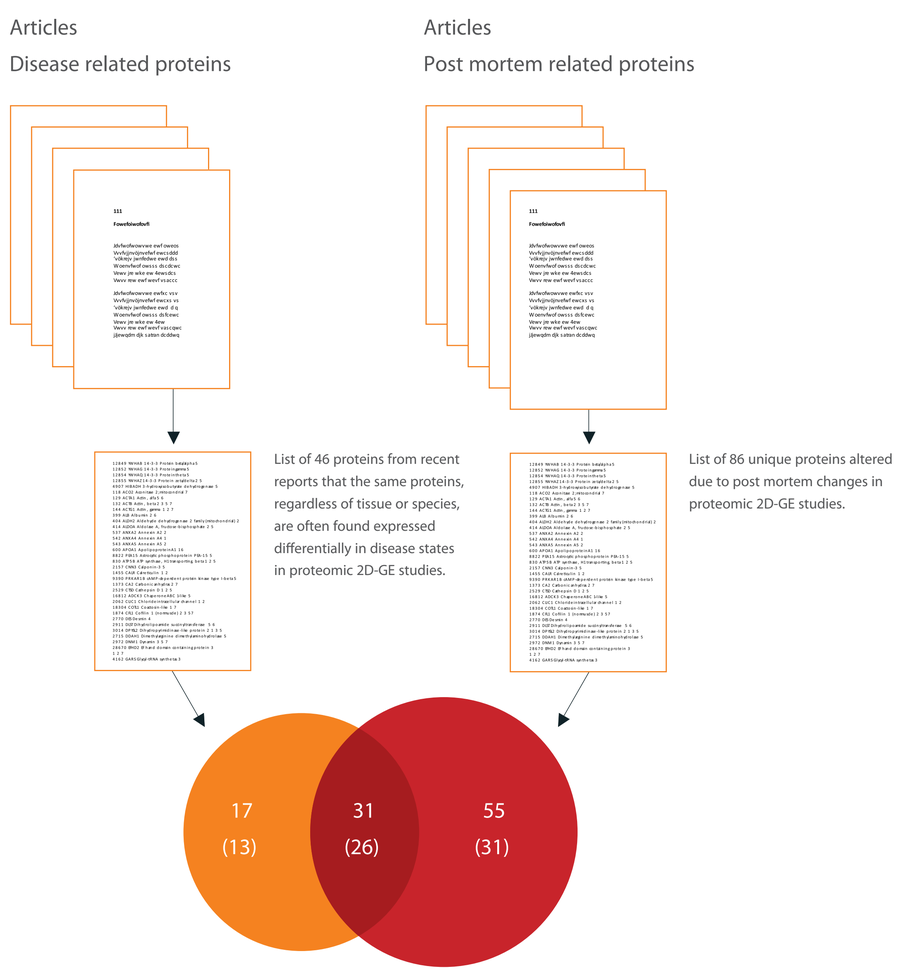

Figure 1. Comparison between the number of proteins that are frequently found as differentially expressed in the literature and the number of proteins that are found to be differentially expressed in studies of post-mortem changes. A remarkably high number (31) of the proteins identified in 2D-GE studies are the same. Numbers in parenthesis are the number of proteins containing phosphorylation sites. Venn diagram, left circle disease markers and right circle illustrates markers of post mortem change.

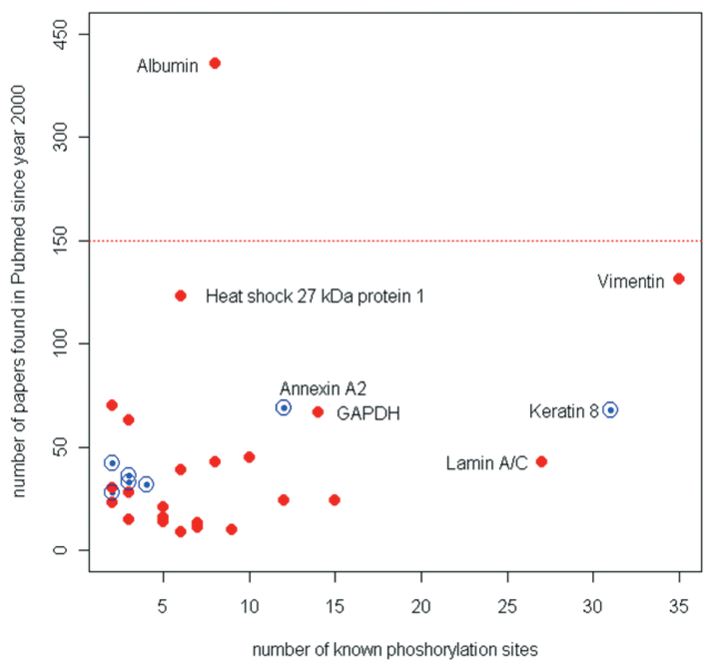

Figure 2. Comparing number of papers found in PUBMED including search terms biomarker/marker or cancer versus number of phosphorylation sites revealed that 30 out of 48 proteins contain at least two sites. If albumin is excluded there is a statistical significant relationship (Pearson correlation coefficient=0.49, r221=0.24, p<0.01) between number of papers found and number of phosphorylation sites. Red dots illustrate protein markers of post mortem changes andblue dots are proteins not reported as markers of post-mortem changes.

Results

The identity of 65% (31 proteins) of the disease-changing proteins subset matched with the post-mortem changing proteins subset. When focusing on phosphorylations 39 (81%) of the 48 disease-changing proteins subset have known phosphorylation sites. In the list of post-mortem changing proteins, 26 of the 31 proteins (84%) contain at least one known phosphorylation site. This over-representation of phosphorylation sites, compared to a total average of ca. 30%, indicates that proteins, and in particular their post-translational modifications, change substantially post-mortem.
Taken together with a recent report of hyperphosphorylation of proteins in non-heat stabilized tissue and the elimination of 99.6% kinase activity in heat-stabilized tissue samples [9], we can conclude that it is highly important to quickly inactivate both phosphatases and kinases to ensure reliable measurement of phosphorylation states without interference from post-mortem events.

Conclusion

We conclude that post-mortem changes, particularly in phosphorylation states, may distort our view of in vivo proteomic profiles. When focusing on analysis of protein phosphorylation states, adequate suppression of both phosphatases and kinases is important. We believe that the use of rapid heat stabilization, as an alternative to conventional snap-freezing, instantly and permanently stops enzymatic activity thereby preventing post-mortem changes to reflect the in vivo status as closely as possible. This approach may help us differentiate true biomarkers from those proteins found in any situation where cells are under stress.

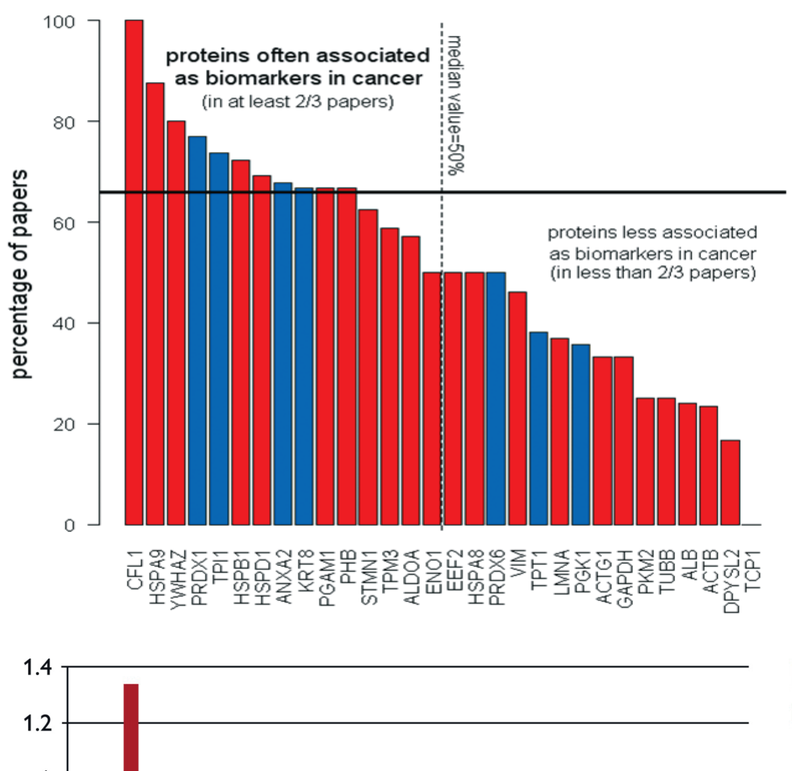

Figure 3. Number of papers found in PUBMED for each protein including the search term "biomarker" with or without cancer association. The median value was found to be 50%. Some proteins such as cofilin 1, HSP70KDa protein (mortalin), 14-3-3 protein zeta/delta and peroxiredoxin 1 are in at least two out of three papers associated with cancer. Red bars illustrates proteins also reported as markers of post mortem changes and blue bars are proteins not reported as markers of post-mortem changes.

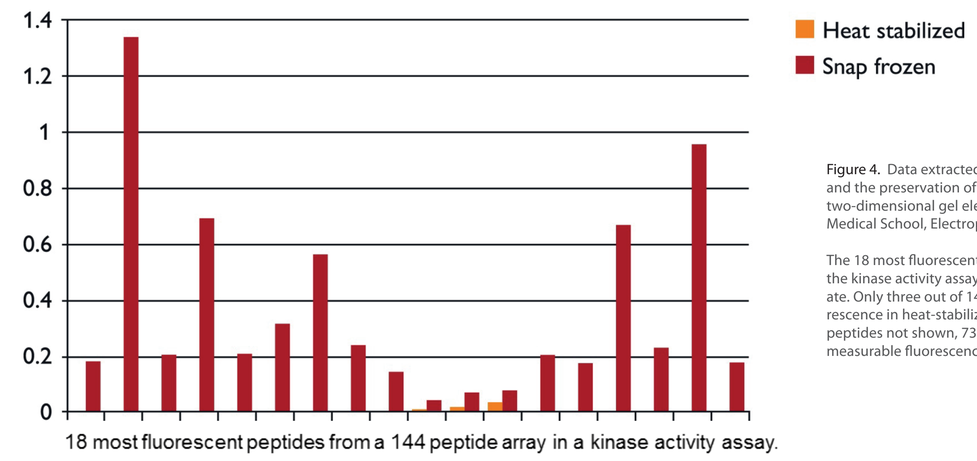

Figure 4. Data extracted from Thermal stabilization of tissues and the preservation of protein phosphorylation states for two-dimensional gel electrophoresis. Smejkal et al., Harvard Medical School, Electrophoresis 2011.

The 18 most fluorescent peptides from a 144 peptide array in the kinase activity assay of frozen, snap-frozen brain homogenate. Only three out of 144 peptides produced measurable fluorescence in heat-stabilized samples. Of the remaining 126 peptides not shown, 73 additional peptide substrates produced measurable fluorescence in snap-frozen homogenates.

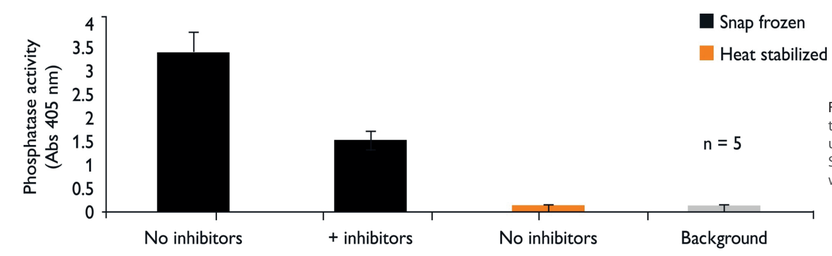

Figure 5. Enzymatic activity in snap-frozen samples compared to heat-stabilized samples. Phosphatase activity was measured using colorimetric pNpp phosphatase kit (AnaSpec). Snap-frozen samples were solubilized in buffers with and without phosphatase inhibitors using microtip sonication.

References

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